a3 cncl.

Asp Ser Glu Glu Asp Glu Glu His Thr Ile Ile Thr Asp Tr Glu Leu Pro Pro [SEQ ID NO: 7]

corresponding to authentic mature TFPI at >90% purity.

REMARKS

The Amendments

Claim 7 has been amended to be in independent form, incorporating the recitations of claim 1. In addition, the preamble of claim 7 has been amended to recite an "[i]solated and purified biologically active TFPI comprising an N-terminal amino acid sequence as shown in SEQ ID NO: 7, wherein the biologically active TFPI has an inhibitory concentration of at least 1 μg/ml in a prothrombin clotting assay" in place of a "factor VIIa/TF/Xa binding protein." This amendment is supported by the specification which discloses that "TFPI produced according to the method of the invention is biologically active." (Page 18, lines 8-9.) The specification also supports this amendment at page 17, lines 24-28 where it discloses that the TFPI purified by the method has an N-terminal sequence that corresponds to SEQ ID NO: 7 and at page 18, line 7 where it discloses that the TFPI purified by the method "displayed an inhibitory concentration in the prothrombin clotting assay of 1 μg/ml."

Claim 7 has also been amended to recite the steps by which TFPI is isolated and purified. The step of transforming yeast cells with a vehicle is supported by the specification where it discloses that "pLACI 4.1 was used to transform three strains of *Saccharomyces cerevisiae*." (Page 16, line 16.) Plasmid pLACI 4.1 comprises a nucleotide sequence that encodes a ubiquitin/TFPI fusion protein. (Page 16, lines 4-5.) The specification also supports the step of

incubating the transformed yeast cells. The specification discloses that factor VIIa/TF/Xa binding proteins are prepared "under conditions favorable for production of the factor VIIa/TF/Xa binding protein, wherein the factor VIIa/TF/Xa binding protein is retained within the yeast cell." (Page 3, lines 20-22.) Claim 7 has also been amended to recite steps of preparing and recovering. These steps are supported by steps (b) and (c) of originally filed claim 1.

The specification has been amended to correct a typographical error on page 17 at line 26. The amendment replaces "[SEQ ID NO: 6]" with"[SEQ ID NO: 7]" immediately following the disclosed amino acid sequence. This amendment is supported by the sequence listing in which the amino acid sequence identified as SEQ ID NO: 7 is identical to the amino acid sequence at page 17, line 26.

None of these amendments introduces new matter.

Priority

A statement setting forth the chain of priority to which the application claims benefit has been added immediately following the title of the invention.

Claim Objections

Claim 7 is objected to as improperly dependent on non-elected claim 1. Claim 7 has been amended to be in independent form, incorporating the recitations of claim 1.

The Rejection of Claim 7 Under 35 U.S.C. § 102(b)

Claim 7 is rejected under 35 U.S.C. § 102(b) as being anticipated by Peterson (*Journal of Biological Chemistry* 266(18):13344-13351). Applicants respectfully traverse.

To reject a claim as anticipated, each element of the claim must be found in a single prior art reference. "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631 (Fed. Cir. 1987). Peterson does not teach every element as set forth in amended claim 7.

Amended claim 7 is directed to an isolated and purified TFPI that is produced in *S. cerevisiae*. The TFPI is biologically active and "has an inhibitory concentration of at least 1 μg/ml in a prothrombin clotting assay." Peterson, as noted in the Office Action, teaches a human TFPI that is purified from *S. cerevisiae*. (Paper 7, page 3, lines 6-7.) Peterson's TFPI, however, is not biologically active with an inhibitory concentration of at least 1 μg/ml in a prothrombin clotting assay.

An unexecuted Declaration under Rule 132 by Dr. Alba Creasey, one of the named inventors, accompanies this amendment[1]. Dr. Creasey's Declaration provides evidence that the biological activity of the TFPI purified by Peterson does not have the biological activity recited in amended claim 7. Dr. Creasey explains that the biological activity of the isolated and purified TFPI disclosed in the subject application is similar to the biological activity of TFPI expressed in and purified from BHK cells. (See Declaration under Rule 132, paragraphs 3 and 12.) This statement is based on experiments performed at Chiron Corporation that demonstrated that the

[1] The executed declaration will be submitted as soon as it is received.

activity of TFPI expressed in and purified from *S. cerevisiae* is similar to the activity of TFPI expressed in and purified from *E. coli*. (See paragraph 4 of the Declaration.) The activity of TFPI expressed in and purified from *E. coli*, in turn, is similar to the activity of TFPI expressed in and purified from C127 cells (Declaration at paragraph 5 and Exhibit 1). Finally, the activity of TFPI expressed in and purified from C127 cells is similar to the activity of TFPI expressed in and purified from BHK cells (Declaration at paragraph 7 and Exhibits 2-4). Thus the activity of TFPI expressed in and purified from *S. cerevisiae* as described in the application is similar to the activity of TFPI expressed in and purified from BHK cells.

The activity of the TFPI purified by Peterson, however, is several-fold lower than the activity of TFPI purified from BHK cells. The activity of Peterson's "[p]artially purified TFPI from yeast showed 5-8 times lower anticoagulant activity than intact TFPI from transfected BHK cells." (Page 13350, column 1, lines 43-46.) The activity of Peterson's TFPI, which is 5-8 times lower than that of BHK cells, is less than the activity of the TFPI described in the present application, which is similar to that purified in BHK cells. Thus, Peterson's TFPI does not have "an inhibitory concentration of at least 1 μg/ml in a prothrombin clotting assay" as recited in amended claim 7. Peterson does not teach each and every element recited in claim 7 and does not anticipate claim 7.

Withdrawal of this rejection to claim 7 is respectfully requested.

Respectfully submitted,

Date: July 29, 2002

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Appendix I. Marked Up Version of the Claims and Specification t Show the Changes Made

Claims

7. (Amended) [The factor VIIa/TF/Xa binding protein] Isolated and purified biologically active TFPI comprising an N-terminal amino acid sequence as shown in SEQ ID NO: 7, wherein the biologically active TFPI has an inhibitory concentration of at least 1 μg/ml in a prothrombin clotting assay, [by the] according to a method [according to claim 1] comprising:

transforming yeast cells with a vehicle, said vehicle comprising a first nucleotide sequence encoding TFPI, wherein the N-terminal amino acid sequence of the TFPI is SEQ ID NO: 7, said first nucleotide sequence being immediately preceded in frame by a second nucleotide sequence encoding ubiquitin, the first and second nucleotide sequences together encoding a fusion protein;

incubating the transformed yeast cells under conditions favorable for production of the TFPI, wherein the TFPI is retained within the yeast cell;

preparing an insoluble fraction of the transformed yeast cells containing the TFPI; and

recovering the TFPI from the insoluble fraction.

French ? or omitting cleavage of ubiquitin from TFPI

Specification

The paragraph at page 17, lines 24-28. Deletions are struck through and additions are underlined.

N-terminal sequencing of the product recovered by this method gave the following correct sequence:

Asp Ser Glu Glu Asp Glu Glu His Thr Ile Ile Thr Asp Tr Glu Leu Pro Pro ~~[SEQ ID NO: 6]~~ [SEQ ID NO: 7]

corresponding to authentic mature TFPI at >90% purity.